V. "Experimental Researches on the Functions of the Mucous Membrane of the Gall-bladder, principally with reference to the Conversion of *Hepatic* into *Cystic* Bile." By GEORGE KEMP, M.D. Cantab. Communicated by the Rev. W. CLARK, M.D., F.R.S., Professor of Anatomy in the University of Cambridge. Received May 1, 1856.

(Abstract.)

Referring to the well-known difference in taste and other physical properties between the bile as it immediately proceeds from the liver and the same fluid after it has been retained for a time in the gall-bladder, the author observes, that the nature of this difference and the agency by which it is effected, are questions which have not yet met with the attention they deserve, and that he had accordingly been led to make them the subject of experimental inquiry. As, however, it is only on rare occasions that the *hepatic* bile can be procured in quantity sufficient for chemical experiment, and then only at the risk of its being altered by pathological conditions of the secreting organ, the author considers that, however clearly individual facts on the subject may be demonstrated, any deductions made therefrom must be referred to the lower department of probable evidence; and it is with this reservation that he lays his conclusions before the Royal Society, whilst, at the same time, he believes that, so far as the nature of the case admits, he has been able to elicit a new fact respecting the mucous membrane of the gall-bladder, which may lead to the better comprehension of the functions of mucous membranes generally.

Assuming, in the first place, that the change in properties which the bile undergoes in the gall-bladder is brought about either by the mucous secretion of that reservoir, or by some operation exerted by its internal membrane, it is observed, with respect to the action of the mucus, 1st, that when left in the gall-bladder in contact with the cystic bile, it is capable of subverting the composition of that fluid. 2nd, That this change is much accelerated by even a moderately elevated temperature. 3rd, That when the contents of the gall-bladder are evaporated to a syrupy consistence, the bile, at first

neutral, becomes alkaline and broken up into several organic groups. 4th, That if the mucus of the gall-bladder be carefully removed by alcohol or acetic acid, and the perfectly fresh bile be then evaporated, these changes do not take place.

From these facts it follows that the mucus of the gall-bladder is a highly catalytic body, and that the analysis of bile which has been left in contact with it, under the conditions above stated, must lead to varying and unsatisfactory results.

From these considerations it naturally occurred to try the effect of placing the mucus of the gall-bladder in contact with hepatic bile; but the experiment was not performed, as it was found impracticable to obtain the mucus of the gall-bladder free from cystic bile without precipitation by reagents. Desiring, however, to ascertain whether the mucus is principally retained in contact with the inner surface of the gall-bladder or diffused through its contents, the author subjected the gall-bladder and its contained fluid, taken from an ox just slaughtered, to a freezing mixture of snow and salt, until all but the central part of the fluid was frozen, and on pouring out the latter found it to contain mucus; thus showing that this secretion is not merely confined to the inner surface of the gall-bladder for the purpose of protection and lubrication of the subjacent membrane, but is diffused throughout the bile contained in that reservoir.

Leaving now the mucous secretion, the object was first to ascertain whether the mucous membrane itself possesses the property of changing the molecular structure of the bile; then to observe whether it possesses any analogy to other mucous membranes, such as the epithelial membrane of the calf's stomach, &c., in acting upon animal fluids and solutions of bodies which readily break up into binary forms; to examine, in the next place, its action upon albumen, the white of an egg being the substance selected; and, finally, to determine its effects on the biliary secretion, as produced in the liver before its admission into the gall-bladder.

*Action of the Mucous Membrane of the Gall-bladder upon Bile.*

December 11, 1855.—The mucous membrane of the gall-bladder was dissected, or rather stripped from the other portion of the viscus, and washed in several waters, until the mucous secretion and bile disappeared; a small portion was now placed in an evaporating dish

and covered with fresh ox-bile, from which the mucus had been carefully removed; the bile also was tested with diluted acetic acid, giving no precipitate or appearance of turbidity. The whole was, at 1 P.M., placed in a warm situation. At 3 P.M. the solution was tested with acetic acid, when it became densely turbid. Temperature, 43°·5 C. The peculiar odour of musk was very distinct in this solution, though not perceptible in the remainder of the fresh bile. A small quantity of water was added, to replace that lost by evaporation, and the solution returned to its warm situation. 6 P.M. again tested, with similar results; the musky odour very strong. 7·30 P.M. again tested, when not only was the solution rendered turbid, but a white precipitate was thrown down; the fluid was also distinctly alkaline.

The mucous lining of the gall-bladder is therefore a catalytic body capable of producing molecular changes in the bile.

*Action of the Mucous Membrane of the Gall-bladder upon certain Bodies which readily break up into Organic Groups.*

*Milk.* December 18.—A portion of mucous membrane was scraped and washed with scrupulous care, in order that the whole of the mucus might be removed; it was then covered with fresh milk and exposed to a temperature of 32° C. In one hour the fluid was separated into serum, turbid with caseine, and an over-stratum of a creamy substance greatly resembling butter, thus exhibiting a catalytic influence very analogous to the action of rennet. The whole contents of the evaporating dish were then set aside, as having effected the object of the experiment; on the 21st, however, the author was induced to taste the fluid, and, to his astonishment, found it intensely bitter, and, what is more remarkable, on applying Pettenkofer's well-known test of sugar and sulphuric acid, with increase of temperature, the characteristic rose-tint was developed. The musky smell was not observable.

*Honey.* December 26.—A portion of mucous membrane, carefully washed, was covered with a solution of honey (one measure of honey to three of water). After six hours' exposure at 38° C., it was found bitter, and gave a precipitate with diluted acetic acid; this was not the case with the original solution. The fluid, being slightly acid, was carefully neutralized with carbonate of soda. The taste,

after another period of six hours, was very bitter. On the 28th the fluid had evaporated down to a thick honey consistence.

The next remark on this subject, in the author's rough notes taken at the time, is the following:—

January 27.—"The honey solution is now nearly evaporated. A mass of crystals (grape-sugar) with a small quantity of syrup, intensely bitter." The syrup could be readily poured off from the crystals. The mucous membrane was not in the slightest degree decomposed; swelled and elastic, not splitting into layers.

It is well known that, after long keeping, granules of grape-sugar are found in honey; therefore, on the 22nd of April, the honey from which the experiments were made was re-examined, and found to be nearly homogeneous and not separated into crystals and syrup; indeed the whole physical appearances are so different from the honey after being operated upon, that the author cannot doubt the influence of the membrane in effecting the changes registered.

At this stage of the inquiry an important doubt suggested itself. In the above experiments no small importance has been attached to the circumstance of bitterness becoming developed in the various solutions when kept in contact with the membrane. In every case indeed the membrane was washed with jealous care, but the fact is palpable, that it is almost impossible to divest the membrane of every trace of bitterness; when this is effected as far as practicable, in a very few minutes the damp membrane increases perceptibly in bitterness. When washed, submitted to pressure between folds of blotting-paper, stretched out on a board and dried as rapidly as possible in a current of warm air, it is still bitter. May not the bitterness alluded to in the above cases be attributed to disintegration of the mucous membrane itself? The following experiments seemed sufficiently simple in their conditions and adapted to answer the query. A body was selected in which well-known molecular disturbances are easily established—cane-sugar.

*Sugar.* December 28.—A portion of membrane was covered with a solution of white sugar; another portion, of the same size, was covered with lukewarm water, and both were exposed to a temperature of 32° C. One hour having elapsed, the watery solution was just perceptibly bitter, the saccharine solution decidedly so.

December 29.—The watery solution was rendered very slightly

turbid on the addition of diluted acetic acid; this reagent, however, threw down a distinct precipitate from the saccharine solution.

January 5, 1856 (from note-book).—"The mucous membrane infused in simple water is today looking disintegrated, in layers, the solution opake and slimy; slightly alkaline, just bitter. The mucous membrane in sugar very bitter, perfectly transparent. I believe that the difference of the mucous membrane, as infused in water and in syrup, appears to be well established."

January 9.—"The sugar solution is perfectly transparent, *very bitter*, very slightly alkaline; the membrane is much swelled out and thickened; *fresh*. The watery solution is becoming decomposed, alkaline, *has lost its bitter taste*, very turbid; the membrane is shrivelled and separating into layers. Microscopic examination referred the turbidness to broken-down epithelium."

The report of the above series of experiments has been thus minutely transcribed, because it seems to place the active agency of the mucous membrane beyond reasonable doubt, so far as the class of bodies alluded to is concerned; but principally because, as will be seen in the sequel, Pettenkofer's method alone appears to fail in some cases as a discriminating test of the bile.

*Action of the Mucous Membrane of the Gall-bladder upon Albumen.*

January 27.—"At 3 P.M. took a portion of dry mucous membrane and carefully washed it in several waters; it was then plunged into the white of an egg. 8 P.M. the glairy fluid is bitter."

January 31.—"The solution apparently increasing in bitterness; a little water added to supply the loss by evaporation."

February 18.—"The albumen solution has from time to time been diluted with water. Today I can barely detect bitterness, nor is the colour changed. On applying Pettenkofer's test, the play of colour, supposed to be characteristic of bile, was very distinct in the fluid portion; the albumen coagulated by the heat, retaining its white colour."

This result was perplexing; on the 19th, therefore, an experiment was made on the white of an egg, *per se*, to ascertain whether the effect was due to the albumen. The white of an egg was first boiled in water, to coagulate the albumen, and the filtered fluid, containing soluble albumen and probably other organic matters, was examined

by Pettenkofer's method. On adding strong sulphuric acid the fluid underwent further coagulation, and the liquid portion became of a beautiful rose colour. It remains to be determined whether the white of the egg contains any of the elements of the bile, or whether Pettenkofer's method fails, as a discriminating test of the bile, in the presence of soluble albumen.

Having thus established the fact that the mucous membrane of the gall-bladder is capable of producing changes on the bodies and under the circumstances above stated, it became an important object of inquiry whether the hepatic fluid proper is capable of being influenced by its contact; but how are we to isolate this secretion? If we take a portion of ox-liver, bruise it down, express the fluid, doubtless containing a large proportion of liver-bile, and place this fluid under an exhausted receiver over sulphuric acid, if in sufficient quantity for examination, it will be decomposed before evaporation is completed. The same fluid undergoes changes also very rapidly at a slightly elevated temperature. It was found in fact that from the temperature of 34° C. to about 60° C. putrefaction is easily produced in ox-liver, whereas, if plunged into water at the boiling-point, no considerable changes of a putrefactive nature occurred. Supposing then we plunge a very thin slice of liver into boiling water, we at once coagulate the albumen, or rather such portion of it as is insoluble in boiling water; we break up the hepatic vessels and obtain a fluid containing a considerable quantity of hepatic bile. It was found better not to keep up the boiling for any lengthened time, as the solution, in that case, contains much soluble albumen. The liquid then having been allowed to boil for a few minutes, was removed from the fire and strained through a cloth; the turbid solution cooled as rapidly as possible; the upper portion poured off from the deposit, and thus experimented upon.

Diluted acetic acid caused no precipitate, nor was any perceptible reaction produced by Pettenkofer's method, which would seem to indicate that the reaction observed in the white of the egg was not occasioned by the presence of albumen.

December 21, 1855.—As in the previous cases, a portion of well-washed mucous membrane was covered with the above solution and exposed to a temperature of 38° C., 39° C. being considered as the maximum temperature of the ox. *In half an hour, on applying*

*Pettenkofer's test, the characteristic colour was beautifully developed.*

December 22.—The solution last alluded to was this morning *distinctly yellow*, very bitter, and formed a precipitate with diluted acetic acid. A musky odour is also perceptible.

On the 21st of December another portion of membrane was covered with the liver-broth and left at the ordinary temperature of the room in which the operation was conducted, 10° C. After thirty hours' digestion, the fluid was in the slightest degree bitter; it was then exposed to a temperature of 50° C. Three hours having elapsed, it was again examined and found decidedly bitter.

December 23.—Cursorily examined the fluid at 4 P.M.; it is noted down as intensely bitter, becoming yellow, with slight musky odour.

December 24.—The solution, just undergoing metamorphosis on December 21st, was this day found as yellow as a diluted solution of ox-gall, musky odour distinct, intensely bitter. Another remarkable feature in common with ox-gall as it is separated from the bladder was now developed: on pouring it into a glass for precipitation with acetic acid, it was found glairy, and instead of running off like water as it did originally, the drops were viscid like a solution of gum-arabic. The bitter taste was now also converted into a sweetish-bitter, identical with the organic matter in ox-bile. The solution gave a dense precipitate with diluted acetic acid, and the peculiar reaction of Pettenkofer's test was most satisfactorily exhibited. It may not be considered unimportant to mention, that, on repeating these experiments a few days ago, a portion of the solution, treated as above, was placed in the hands of a bystander wholly ignorant of the matter, with a request to smell without looking at it; the report was, "You are mixing up some indian ink;" indeed, the odour of musk seems to be one of the most important conjunctive indications of the presence of bile, after a few hours' exposure to atmospheric air.

Many more experiments are registered in the author's rough notes; some of these have been repeated within the last few days, all with confirmatory results; the following generalizations therefore appear legitimately deduced from the research:—

1st, That the mucus of the gall-bladder is not merely a secretion destined to lubricate the interior of that organ and protect it from

the irritation of its other contents, but is an essential integral portion of the cystic bile.

2ndly, That the gall-bladder is not merely a receptacle and reservoir for the bile, but an organ highly endowed with organic functions; and that the proper secretion of the liver is converted into cystic bile mainly through the agency of its mucous membrane.

In thus breaking up the surface of an interesting field of research, the writer is fully aware that a great amount of labour must still be expended upon its development; he would also be understood to regard these experiments merely as expressing the results of non-vital reactions. We can hardly indeed doubt that, under the influence of vitality, acting through the medium of that most important department of the nervous system, the *solar plexus*, molecular changes, not improbably analogous to or identical with those which we have described, may be carried on with an energy and efficiency which we cannot hope to witness in the laboratory. Professor Clark, of Cambridge, has already suggested the extension of the research to the case of animals which have no gall-bladder, and in which the hepatic secretion is at once poured into the duodenum, to take its part in the process of assimilation. The conjecture may not be far from the truth, that the mucous lining of the intestinal canal, the parotid gland, the pancreas, the kidney, the urinary bladder, has each its specific predestined function to perform; and that in working out the subject, we may fall upon many a useful fact, many a beautiful analogy, and much to supply the wants and alleviate the sufferings of man.

Thursday the 29th of May having been set apart for the celebration of the Peace, the President announced that the next ordinary meeting of the Society would be held on Thursday, the 12th of June,